PB82-207846

Rosette Glands in the Gills of tne Grass Shrimp, 'Palaemonetes pugio' I. Comparative Morpnology, Cyclical Activity, and Innervation

University of West Florida
Pensacola

Prepared for

Environmental Research Lab.
Gulf Breeze, FL

1982

U.S. Department of Commerce
National Technical Information Service
NTIS

ERL,GB 0225

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/J-82-150	2. Journal Article
	PB82-207846
4. TITLE AND SUBTITLE: ROSETTE GLANDS IN THE GILLS OF GRASS SHRIMP, PALAEMONETES PUGIO. I. COMPARATIVE MORPHOGOLOGY, CYCLICAL ACTIVITY, AND INNERVATION	5. REPORT DATE: April 1982
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): Daniel G. Doughtie and K. Ranga Rao	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Faculty of Biology University of West Florida Pensacola, FL 32504	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO. R807417
12. SPONSORING AGENCY NAME AND ADDRESS: U.S. Environmental Protection Agency Environmental Research Laboratory Office of Research and Development Gulf Breeze, FL 32561	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE: EPA/600-

15. SUPPLEMENTARY NOTES: JOURNAL OF MORPHOLOGY 171:41-67 (1982)

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

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NATIONAL TECHNICAL
INFORMATION SERVICE
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SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT: Release to public	19. SECURITY CLASS *(This Report)*: unclassified	21. NO. OF PAGES: 29
	20. SECURITY CLASS *(This page)*: unclassified	22. PRICE

EPA-600/J-82-150
Journal Article

JOURNAL OF MORPHOLOGY 171:41-67 (1982)

Rosette Glands in the Gills of the Grass Shrimp, *Palaemonetes pugio*. I. Comparative Morphology, Cyclical Activity, and Innervation

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ABSTRACT Two types of exocrine rosette glands (called type A and type B), located in the gill axes of the grass shrimp *Palaemonetes pugio*, are described. The type A glands are embedded within the longitudinal median septum of the gill axes, whereas the type B glands typically project into the efferent hemolymph channels of the gill axes. Although both glands have certain common characteristics (i.e., a variable number of radially arranged secretory cells, a central intercalary cell, and a canal cell that forms the cuticular ductule leading to the branchial surface), they differ in the following respects. The type B gland is innervated, but the type A gland is not; axonal processes, containing both granular (ca. 900–1300 Å) and agranular (ca. 450–640 Å) vesicles, occur at a juncture between adjacent secretory cells and the central cell of the type B gland. The secretory cells of type A and type B glands differ in their synthetic potential and membrane specializations. These differences are more pronounced in well-developed, mature glands, most frequently encountered in larger (24–28 mm, total length) grass shrimp, than in the underdeveloped, immature glands that are most abundant in smaller (14–18 mm, total length) grass shrimp. Thus, in mature glands, the secretory cells of the type A rosette glands are characterized by extensive RER, abundant Golgi, and numerous secretory granules, whereas the secretory cells of the type B gland are characterized by extensively infolded and interdigitated basal plasmalemmas and by the presence of numerous mitochondria. In general, both types of glands exhibit increased secretory activity soon after ecdysis. The central and canal cells in both glands seem to have a role in the modification of the secreted materials. The possible functions assigned to the type A gland and the type B gland include phenol-oxidase secretion and osmoregulation, respectively.

Allen (1893) first described the occurrence of two types of rosette glands, the "clear" and "reticulate" glands, in the gills of the shrimp, *Palaemonetes varians*. Since then, many investigators have reported on the occurrence, morphology, and functional aspects of rosette type glands in the subcuticular dermal tissues of Crustacea using both light microscopy (Yonge, '32; Gorvett, '46; Richards, '51; Stevenson, '61, '64; Skinner, '62) and electron microscopy (Elofsson et al., '78; Arsenault et al., '79). Although some of these investigators have noted changes in the secretory activity of the rosette glands (e.g., Yonge, '32; Gorvett, '46; Stevenson, '61; Arsenault et al., '79), they did not report any morphological diversity in the rosette glands studied. This raised the question of whether the two types of rosette glands described by Allen (1893) were inherently distinct, or whether they represented different phases of secretory activity of a single gland type. In order to answer this question, we have examined the ultrastructure of the rosette glands in *Palaemonetes pugio*, utilizing specimens of various sizes and in various stages of the molt cycle.

The results of our study will be reported in two parts. The present paper shows that two types of rosette glands are indeed distinguishable by electron microscopy, and describes the morphological features, as well as the molt-related secretory activities of these glands. This report also shows that one of these gland types is unlike any of the previously described crustacean exocrine glands in that it is innervated and has characteristics of transport epi-

thelia. The second paper of this series describes the events associated with premolt ductule reformation in the rosette glands.

MATERIALS AND METHODS

Grass shrimp, *Palaemonetes pugio*, measuring 14–28 mm (total length), were collected from grass beds in Santa Rosa Sound, Gulf Breeze, Florida. Shrimp were maintained individually in glass jars containing seawater (10‰ salinity) at a temperature of 20 ± 1°C under 12-hour light–12-hour dark conditions in the laboratory. The shrimp were examined daily for ecdysis. The progress of shrimp through the molt cycle was assessed by examining the uropods and telson of live shrimp at 100 × magnification under a binocular compound microscope. The incidence of apolysis and the progress in neosetogenesis in the uropods were used as the criteria for identifying the stages (Drach and Tchernigovtzeff, '67; Conklin and Rao, '78) in the molt cycle of the shrimp to be sacrificed for electron microscopy.

The shrimp were placed into cold (4°C) 4.5% glutaraldehyde buffered with 0.2 M *s*-collidine, pH 7.4, in a modification of Bell's fixative (Bell et al., '69), and the carapace was removed. The pleurobranchiate gills situated above the five pairs of pereiopods were then dissected and sliced into smaller pieces. These were fixed at room temperature in *s*-collidine-buffered glutaraldehyde for 3 hours followed by overnight fixation in the cold (4°C). The tissue was then washed in 0.2 M *s*-collidine buffer and postfixed in cold 2% OsO_4 for 4 hours, after which the tissue was again washed in 0.2 M *s*-collidine buffer and then stained en bloc with 7% aqueous uranyl acetate for 15 minutes. Dehydration was carried out in a graded series of ethanols, followed by propylene oxide. The tissue was finally embedded in Epon 812 in flat molds and polymerized at 60°C. Thin sections with gold and silver interference colors were cut on an LKB Ultrotome II, using glass knives, and mounted on uncoated and Formvar-coated copper grids. These were stained with lead citrate and viewed with a Philips 201 electron microscope. Thick sections (2–3 μm) were cut from Epon-embedded material, stained with toluidine blue, and viewed with a light microscope for orientation.

RESULTS

Two types of rosette glands occur in the pleurobranchiate gill axes of the grass shrimp, *Palaemonetes pugio*. These will be referred to as type A and type B glands. In smaller grass shrimp (14–18 mm, total length) the majority of these two types of glands appear relatively underdeveloped or immature, whereas in larger grass shrimp (24–28 mm, total length) most of the rosette glands of both types appear fully developed or mature. The mature glands are described first, and are diagrammatically outlined in Figure 1.

Mature type A rosette glands

Location and general morphology. These glands (50–125 μm in diameter) usually occur at regular intervals within the entire length of

Abbreviations

A, Type A rosette gland
a, gill axis
Af, afferent hemolymph channel
ax, axon
be, new branchial exoskeleton
bm, basement membrane
bs, branchial surface
c, cuticle of cephalothorax
C, central cell
CC, canal cell
ch, extracellular channels of central cell
d, ductule
ec, extracellular channels of type B gland secretory cell
EC, epithelial cell
es, ecdysial space
f, fibrillar elements
g, Golgi
gb, granule-like bodies
gf, granular and filamentous material
i, intima of vent
l, gill lamella
m, mitochondria
ma, macula adherens
mi, microvilliform plasmalemma
msa, median axial septum
mt, microtubules
n, nerve fascicle
nd, new ductule
nv, neurosecretory vesicle
obe, old branchial exoskeleton
od, old ductule
p, peduncle
r, reservoir
rer, rough endoplasmic reticulum
s, synapse
sa, secretory apparatus
SC, secretory cell
sd, septate desmosome
ser, smooth endoplasmic reticulum
sg, secretory granule
t, axon terminal
ter, smooth tubular endoplasmic reticulum
v, vent of central cell
vs, vesicles
vs_1, forming vesicle
vs_2, secreted vesicle

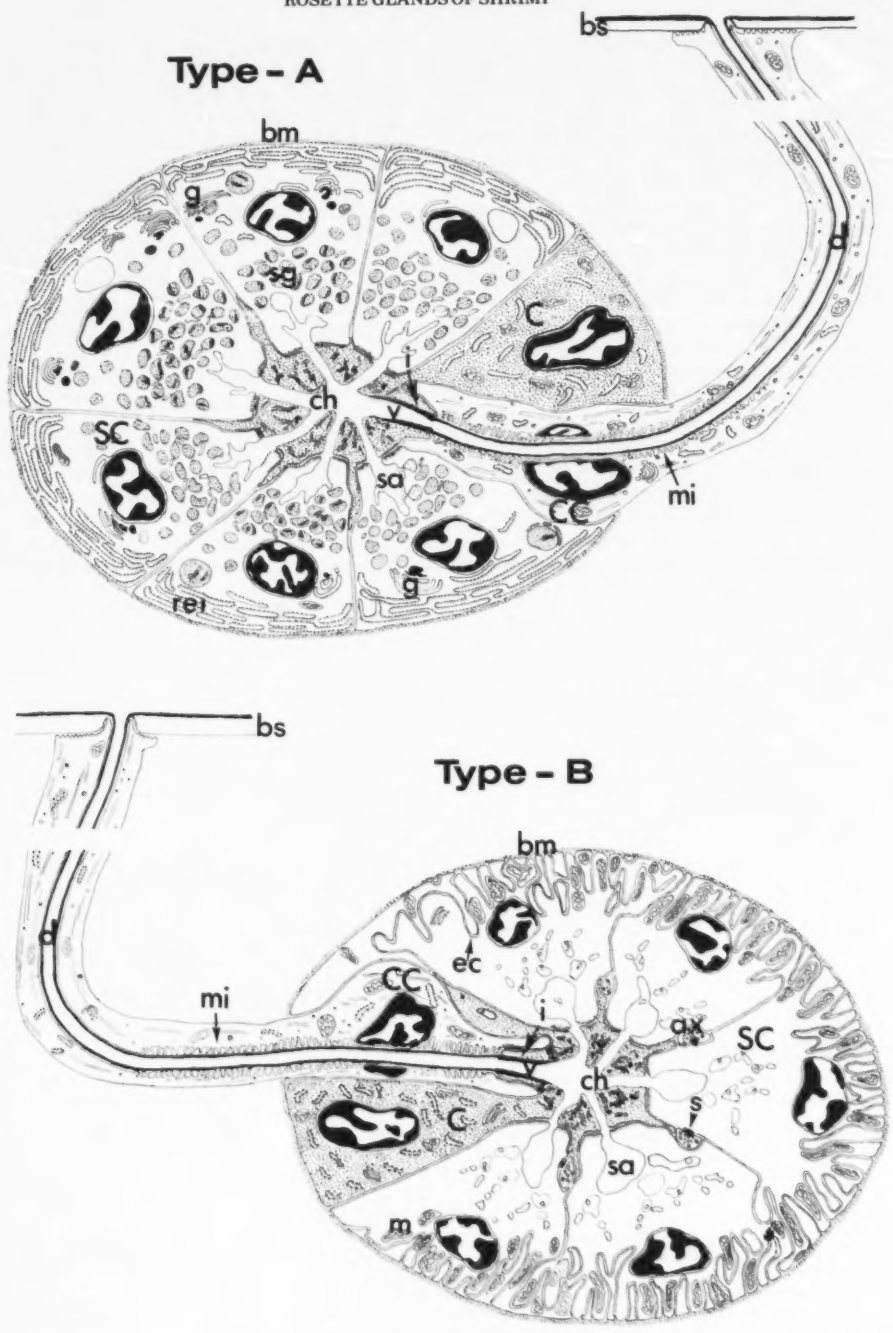


Fig. 1. Schematic diagrams of the type A and type B rosette glands that occur in the gill axes of the grass shrimp, *Palaemonetes pugio*.

the longitudinal median septum of the gill axes (Fig. 2). The distance between adjacent glands is approximately equal to the distance between adjacent gill lamellae (ca. 30–35 μm). The mature glands (Figs. 1, 3) are usually spherical or ovoid and consist of 14–17 radially arranged secretory cells, a central cell, and a canal cell. The apices of the nearly conical secretory cells and the canal cell are directed toward the center of the gland, where they are enveloped by the polarized cytoplasm of the central cell. The nucleus of the latter is located near the gland periphery. A system of extracellular channels, formed by the central cell, communicates with the secretory apparatus of each secretory cell and also with the ductule of the canal cell (Fig. 1). A granular basement membrane covers those surfaces of the type A gland that are not in direct contact with the cells of other glands, or connective tissue.

The basal cytoplasm of each secretory cell contains a usually ovoid or spherical nucleus, RER, Golgi apparatuses and occasional mitochondria (Fig. 1). The rough endoplasmic reticulum is extensive (Figs. 1, 3), particularly during early premolt (stages D_0–D_1). The numerous Golgi bodies possess as many as ten saccules. The lumina of the outer Golgi saccules, near the forming face, are electron-lucent, whereas the lumina of the inner Golgi saccules are constricted and contain an electron-dense material. Nearly spherical, membrane-delimited masses of electron-dense material are frequently situated within the concavity formed by the inner Golgi saccules (Fig. 4). Attenuated connections between the latter and the dense masses are occasionally observed, and it is presumed that this association represents an initial stage in granule formation. Throughout most of the molt cycle, the apical and median cytoplasm is densely populated with large (0.8–1.2 μm) secretory granules (Figs. 1, 3) that are interspersed with numerous, inconspicuous cisternae of smooth endoplasmic reticulum as well as occasional cisternae of rough endoplasmic reticulum. The

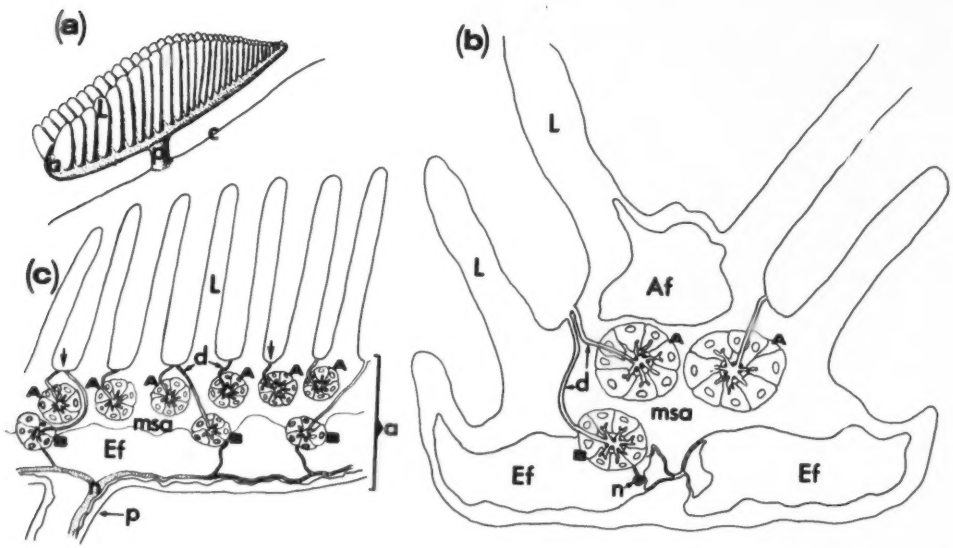


Fig. 2. a) Schematic diagram of a pleurobranchiate gill of the grass shrimp, *Palaemonetes pugio*. The gill is attached to the cephalothorax by a stalk-like peduncle, and consists of a long axis to which two rows of lamellar plates are attached. b) Schematic representation of a pleurobranchiate gill from a grass shrimp, in cross section. Both the type A and type B rosette glands are located within the longitudinal median septum of the gill axis. The type B rosette gland frequently projects into the efferent hemolymph channel and is always innervated by a nerve axon. c) Schematic diagram of a grass shrimp pleurobranchiate gill in sagittal section, showing the regularly spaced type A glands and the more randomly spaced type B glands. Note that the cuticular ductules of both gland types terminate at the branchial surface at points midway between adjacent lamellae (arrows).

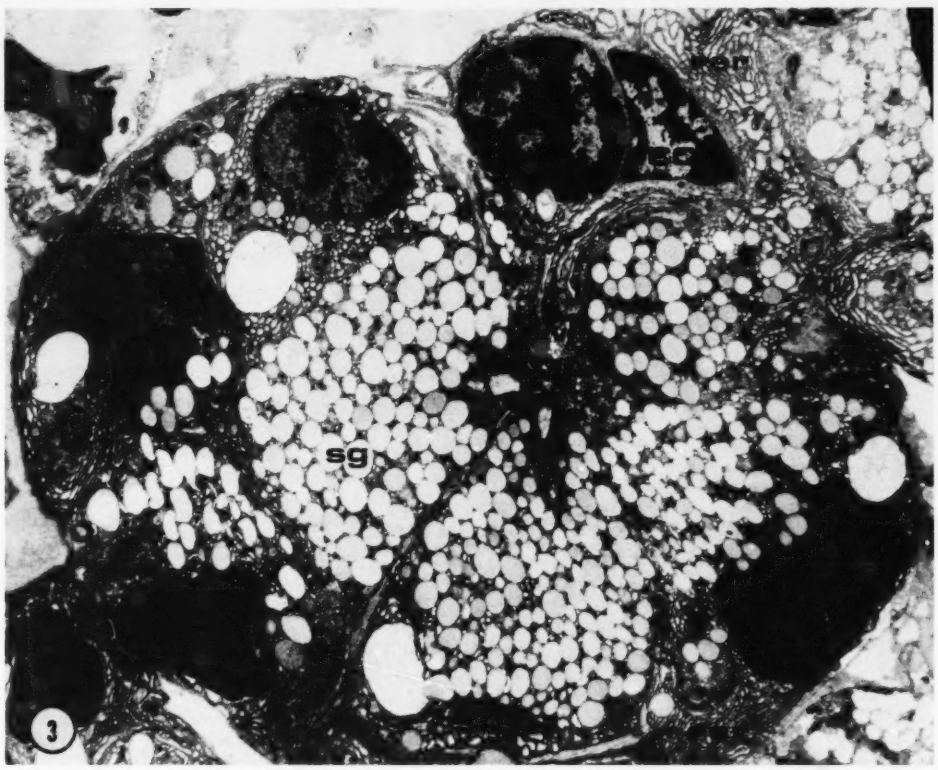
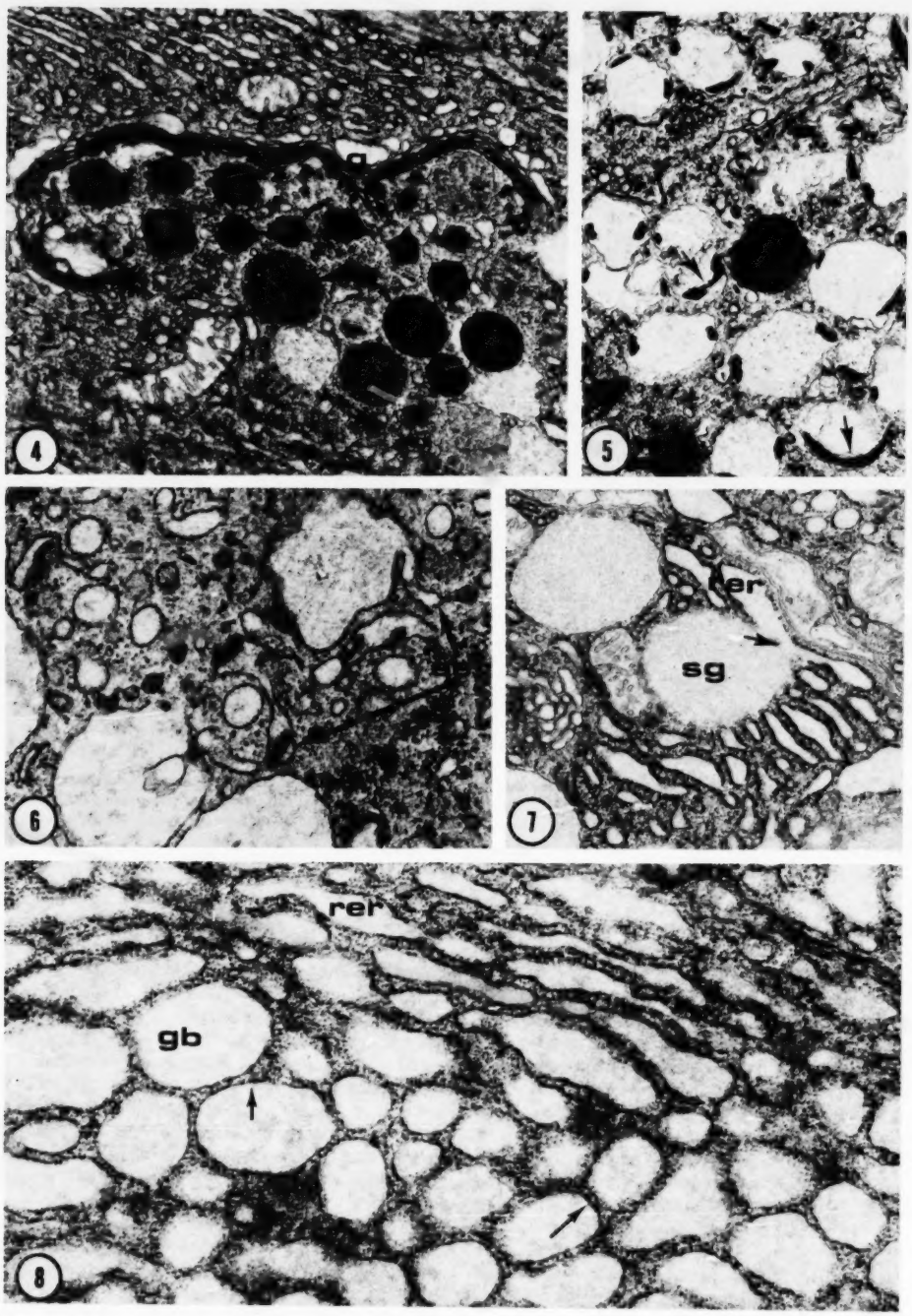


Fig. 3. Overview of mature type A rosette glands during premolt. The secretory cells show a characteristic abundance of secretory granules (sg) and the basal perinuclear cytoplasm contains an extensive rough endoplasmic reticulum (rer) and numerous Golgi apparatuses (g). The central cell (C) and canal cell (CC) are also visible as well as the vent (v) of the former. × 2,400.

contents of the granules are variable but usually consist of a moderately electron-dense, fine-grained or filamentous material suspended in a lucent matrix. During late premolt (stages D_3–D_4), electron-dense lamellar inclusions are present in many granules (Fig. 5). The dense inclusions appear to be formed within the cisternae of smooth endoplasmic reticulum and then transported directly from the cisternae to the granule matrices (Fig. 6). Occasionally, secretory granules occur in close apposition to cisternae of rough endoplasmic reticulum and there appears to be confluence between the granule matrix and the lumen of RER cisternae (Fig. 7). Spherical bodies containing a finely granular and filamentous material, which resemble secretory granules, commonly occur in the vicinity of the extensive rough endoplasmic reticulum of the basal cytoplasm (Fig. 8). The delimiting membrane of the smaller granule-like bodies is usually studded with ribosomes. In the case of the larger granule-like bodies, however, the ribosome coating is very sparse (Fig. 8). It appears then that the lamellar cisternae of rough endoplasmic reticulum may evolve spherical, ribosome-studded bodies which in the process of enlargement lose their ribosome coating so as to resemble closely (and perhaps become) secretory granules.

The secretory apparatus is merely a tubular extracellular channel formed from the simple invagination of the apical plasmalemma (Fig. 9). The contents of the secretory granules are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exocytotically discharged into the secretory apparatus and exit through a small (≅ 0.5 μm) orifice into the extracellular channels of the adjacent central cell (Figs. 1, 9). A macula adherens (0.3 μm) and septate desmosome (0.2 μm) surround the orifice and bind the secretory cell plasmalemma with that of the adjacent central cell. Septate junctions do not occur between the opposing membranes of adjacent secretory cells.

Central cell. The cytoplasm of the central cell may be viewed as bilobed and consisting of a central and peripheral lobe with respect to the whole rosette gland (Fig. 1). The peripheral lobe contains the nucleus, occasional cisternae of rough endoplasmic reticulum and Golgi, and mitochondria. The nucleus (≅ 8.5 μm) is usually smaller than the nuclei of adjacent secretory cells, and the perinuclear cytoplasm is relatively electron-dense. The central cytoplasmic lobe envelops an anastomosing system of extracellular channels that connect the secretory apparatuses of the secretory cells with the duct of the canal cell (Figs. 1, 9). These channels constitute a simple hole through the central cell, as there are no oposing plasmic surfaces. The cytoplasm surrounding the extracellular channels is laced with electron-dense bundles of granular and filamentous material (Figs. 1, 9, 21) and contains vesicles (≅ 0.1 μm) with moderately dense matrices.

The extracellular channels, leading away from the secretory apparatuses, all merge into a common cylindrical vent (1.5–2.5 μm long × 1.0 μm wide). The vent (Figs. 9, 10), together with a thin sheet of enveloping central cell cytoplasm (Fig. 10), appears as a small hill-like protuberance. A thin intima, formed by the central cell, lines the vent (Figs. 10, 11) and is composed of a moderately dense granular material (Fig. 11). As the intima ascends along the vent walls, its thickness increases gradually (maximum thickness ≃ 65 nm) until it merges with the much thinner basal region of the cuticular ductule, formed by the canal cell (Fig. 11). The plasmalemmas of the canal cell and central cell are joined adjacent to the vent base by a macula adherens (0.5 μm wide) and a septate desmosome (0.3 μm wide).

Canal cell and the ductule. Typically situated between the peripheral lobe of the central cell and a secretory cell, the canal cell possesses a generally polymorphic nucleus that is located near the gland periphery (Figs. 1, 3). A cylindrical extension of the canal cell (≅ 2.5–4 μm diameter), which ensheaths the ductule (0.8–1.8 μm diameter), leaves the rosette gland, and travels an irregular path (≃ 125 μm in length) to the branchial surface (Figs. 1, 14, 15). The canal cell cytoplasm is characterized by occasional Golgi bodies, mitochondria, and cisternae of rough endoplasmic reticulum, as well as abundant microtubules and semilucent vesicles (0.1–0.25 μm). The latter two are the predominant constituents of the cylindrical extension leading to the cuticular surface.

The walls of the ductule are composed of a trilaminated epicuticle (23 nm, thick) (Figs. 11, 15), which is continuous with the other epicuticle of the branchial exoskeleton. Typically the ductule appears to be slightly folded so that a furrow occurs in the ductule wall over its entire length. For this reason, cross sections of the ductule usually reveal an expanded U-shaped profile (Fig. 16), whereas sagittal sections

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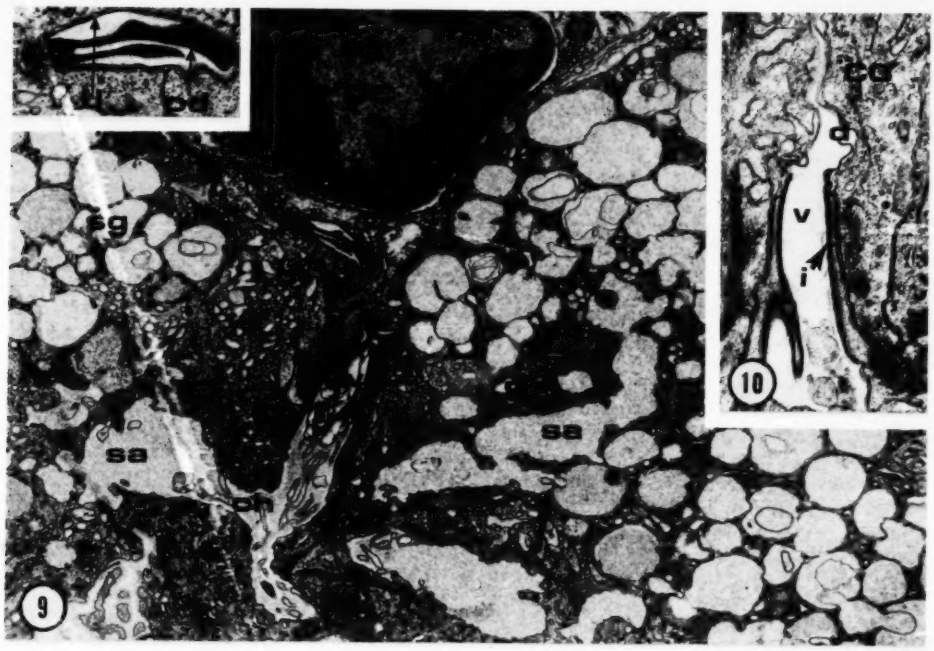
Fig. 4. High magnification of a Golgi apparatus (g) in the secretory cell basal cytoplasm during premolt. Notice the lucent nature of the outer saccules and the close association of numerous secretory granules (sg) with the dense inner Golgi saccules. × 20,000.

Fig. 5. Micrograph depicting the presence of electron-dense inclusions (arrows) within the secretory granules of a type A rosette gland during late premolt. × 10,000.

Fig. 6. Secretory granules of a type A rosette gland during late premolt, which are associated with cisternae of the smooth endoplasmic reticulum (ser). Notice the dense inclusions in the latter; these are presumably transported directly to the granule matrix. × 30,000.

Fig. 7. Frequently, the matrices of type A rosette gland secretory granules (sg) appear to be confluent (arrow) with the lumen of rough endoplasmic reticular cisternae (rer). × 24.000.

Fig. 8. High magnification of the basal cytoplasm of a type A secretory cell, similar to those demonstrated in Figure 3, which shows numerous lamellate cisternae of rough endoplasmic reticulum (rer). A number of spherical secretory granule-like bodies (gb), which appear to have arisen from the rough endoplasmic reticulum, are also present. Notice that some of these granule-like bodies are still studded with small patches of ribosomes (arrows). × 25,000.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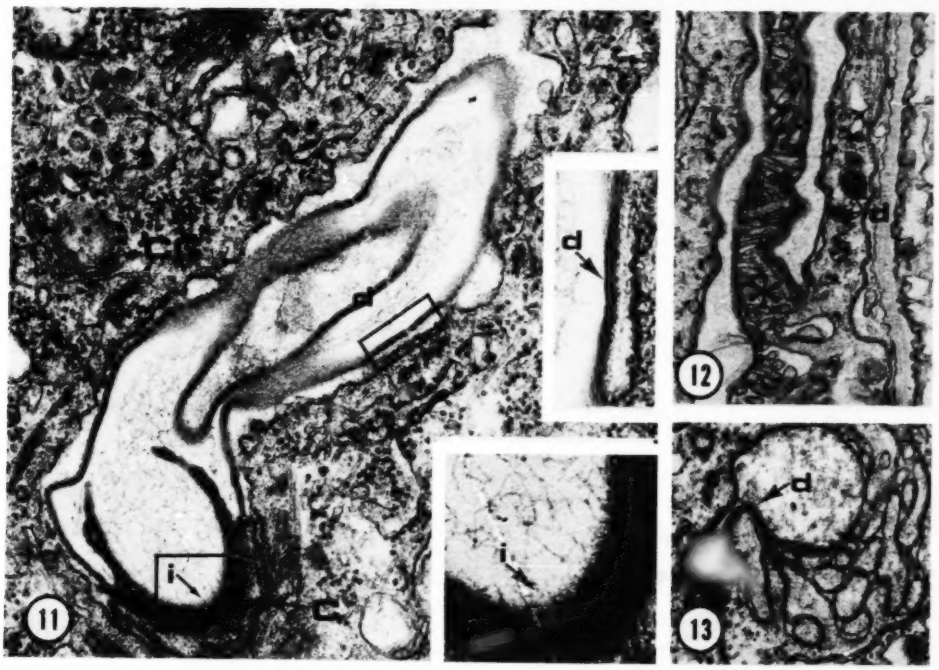
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wrongly imply the presence of two parallel ductules (Fig. 12). The terminal orifice of the ductule ($\simeq$ 0.8 μm wide) is located at the cuticular surface of the gill axis (Fig. 14) at points midway between adjacent gill lamellae (Fig. 2).

The canal cell wraps around the ductule to form an extracellular canal. Where the opposing plasmic surfaces meet, they are joined by both macula adherens and septate desmosomes (Fig. 16). Within the main body of the rosette gland and occasionally in regions a considerable distance away from the gland, the plasmalemma of the canal may be thrown into folds or microvilliform extensions of variable dimension. These folds or microvilli usually project into the ductule furrow (Figs. 12, 13, 15, 16) and contain a loosely woven filamentous material (Fig. 13).

Secretory activity. The secretory granules, as indicated above, are formed in association with the Golgi and endoplasmic reticulum. The release of the granule contents occurs near the cell apex as the granules fuse with each other, and with the secretory appratus. During intermolt the secretory activity of type A rosette glands is sporadic; in fact, in many glands, the ductule appears collapsed over much of its length, indicating a temporary cessation of secretion (Fig. 17). Secretory activity is also negligible during early premolt (stages D_0–D_1) at which time the lumen of the ductule is occluded by numerous cytoplasmic processes, originating from the secretory cells, which presumably contribute to the formation of a new cuticular ductule (see Doughtie and Rao, '82). However, during early premolt, the secretory cells are engaged in amplified synthetic processes as evidenced by the proliferation of RER and accumulation of secretory granules in the cell apices. During late premolt, after the new cuticular ductule is formed around the old ductule and the cytoplasmic processes have degenerated, secretory activity by the gland is resumed. Typically the secretion ascends between the old and the new ductule (Fig. 18) to the ecdysial space, causing a bulge in the latter (Fig. 20). Occasionally, however, the secretion may ascend to the branchial surface via the old ductule (Figs. 9, 19). The secretory exudate is relatively dense and appears as a fine-grained precipitate (Fig. 20).

Immediately after ecdysis, the apical and median cytoplasm of the secretory cells is replete with mature secretory granules containing a sparse filamentous material as well as occasional eccentric patches of a moderately dense material suspended in a lucent matrix. The granules fuse with the secretory apparatus, and their contents pass into the extracellular channels of the central cell. Upon entering the latter, a conspicuous morphological transformation in the nature of the secretion frequently occurs. Specifically, the secretion changes from a moderately lucent material to one that is extremely electron-dense (Fig. 21). This change is sometimes delayed until the secretion passes into the ductule of the canal cell (Fig. 9). Whether there is a chemical basis for the observed change is not known.

Twenty-four hours after ecdysis the secretory cells exhibit a marked reduction in the numbers of contained secretory granules, and secretory activity appears relatively subdued (Fig. 22).

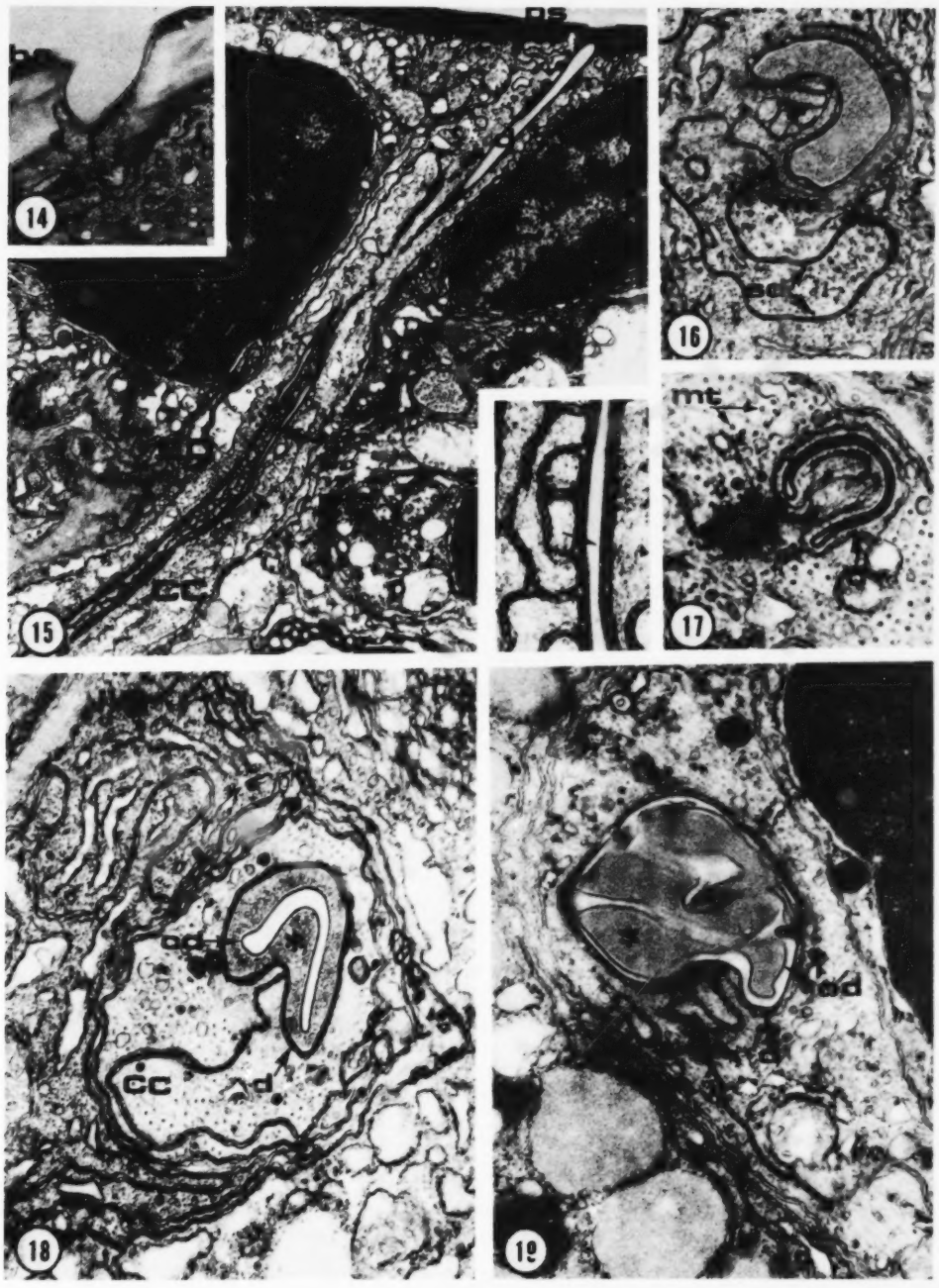
Fig. 9. Overview of central core of type A rosette gland during late premolt. The secretory granules (sg) appear to fuse (arrow) with the secretory apparatuses (sa) at the apices of the component secretory cells (SC). The contents of the granules then pass through the extracellular channels (ch) and vent (v) of the central cell (C) toward the ductule (d) of the canal cell (CC). Sinuous patches of an electron-dense material (*) typically surround the extracellular channels. × 7,200. Inset: Within the lumen of the ductule (d), shown here at a higher magnification, resides the old ductule (od), which was formed during the previous premolt period and which will be sloughed at ecdysis. Notice the extremely dense nature of the secretory material contained within the lumen of the old ductule. × 18,500.

Fig. 10. Micrograph of vent region (v) formed by type A rosette gland central cell as seen during intermolt. Notice that the intima (i) of the vent gradually increases in thickness until it merges with the ductule (d) of the canal cell (CC). × 12,000.

Fig. 11. High magnification of the region where the intima (i) of the central cell (C) merges with the basal region of the ductule (d) in the canal cell (CC). × 42,000. Insets: Higher magnifications of the enclosed regions showing the trilaminar organization of the basal ductule cuticle (d) and the granular appearance of the central cell intima (i). Both × 96,000.

Fig. 12. Sagittal section through the basal region of a typically furrowed type A gland ductule (d) demonstrating the extent to which the opposing canal cell plasmalemma may be infolded or microvillate (*). × 16,000.

Fig. 13. A cross section through the furrowed basal ductule (d), demonstrating the presence of a filamentous material (*) within the microvilliform extensions of the canal cell plasmalemma. × 16,000.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Mature type B rosette glands

Location and general morphology. The distribution of the type B rosette glands is more random than that of the type A glands. Typically, these glands are partially embedded within the branchial tissues that surround the efferent hemolymph channels (Fig. 2). Furthermore, as much as 70% of the gland may project into the latter. B type glands may be spherical, but are more frequently ovoid (Figs. 1, 23). Mature glands vary in size (45–70 μm) and volume in relation to their secretory activity. The glands are composed of 10–14 conical secretory cells, a central cell, and a canal cell. The morphological relationships between these cells are much like those that charcterize the type A rosette glands (Fig. 1). The major differences between the two gland types lie in their location, the structure of their component secretory cells, and the fact that the type B rosette glands are innervated (Fig. 1). For this reason, the latter two characteristics will be described in detail, and the description of the canal and central cells will be kept brief.

Secretory cells. These nearly conical cells are radially arranged with the apices directed toward the gland center, where they are enveloped by the cytoplasm of the central cell. The secretory cell cytoplasm may be differentiated into an apical and basal zone (Fig. 1). Residing in the basal zone are the ovoid or spherical nucleus, numerous ribosomes, mitochondria, occasional cisternae of ER, and extremely rare Golgi elements. The gland surface is overlaid by a basement membrane (Figs. 24–26) of variable fibrous or finely granular constitution. The underlying basal plasmalemma, particularly in those regions that face the hemolymph, is thrown into numerous folds (Fig. 27), which may extend as deeply as 15 μm into the subjacent cytoplasm and occasionally beyond the level of the nucleus. The concavities of these typically narrow folds contain the cytoplasmic extensions of adjacent secretory cells (Fig. 28). This interdigitation between adjacent secretory cells brings about the formation of narrow (ca. 200 Å), extracellular channels that appear to open only at the glandular surface. Oblong or spherical mitochondria thickly populate the cytoplasm associated with these folds (Fig. 27). There are no junctional specializations within the intercellular channels, and high magnification micrographs did not demonstrate that the infolded membranes possessed a coating of particles or exhibited assymetry. Pinocytotic invaginations of the basal cell surface, adjacent to the basal lamina, are uncommon.

The apical cytoplasm, situated between the nucleus and the cell apex (Fig. 1), appears more electron-dense than the basal zone. It contains very few mitochondria and no golgi bodies, but does contain a network of smooth, tortuous, tubular (ca. 100 nm diameter) endoplasmic reticulum (TER). This network is displaced by

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Fig. 14. Site where the ductule (d) terminates at the branchial surface (bs). × 15,000.

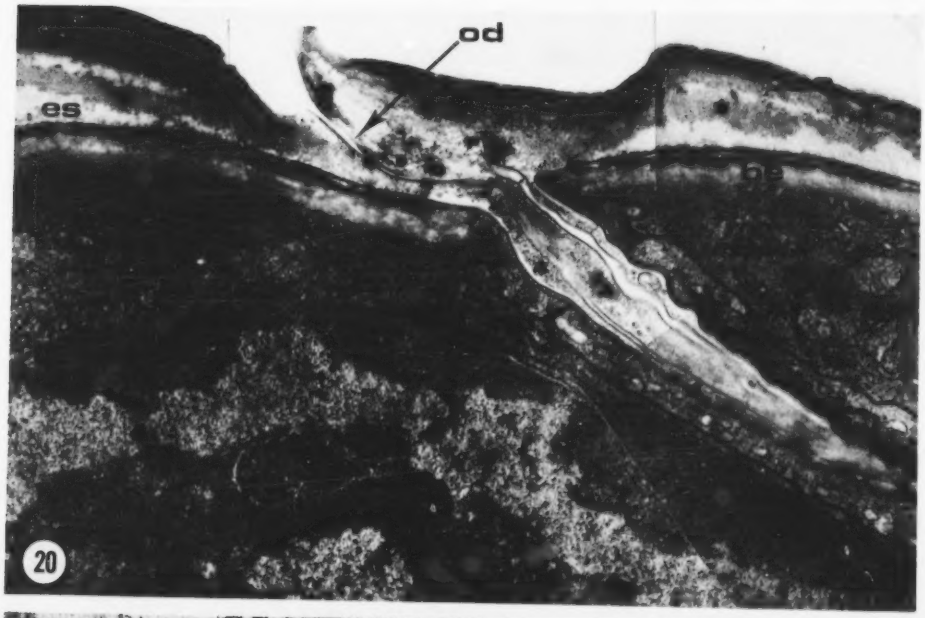
Fig. 15. Low magnification sagittal section of the apical region of the ductule (d) as well as the enveloping, relatively electron-lucent canal cell cytoplasm (CC), ascending between two epithelial cells (EC) toward the branchial surface (bs). The canal cell plasmalemma, adjacent to the concavity of the furrowed ductule, is microvilliform (arrow). × 6,300. Inset: Higher magnification of ductule at site near the arrow, showing that the apical region of the ductule is composed of trilaminated epicuticle (arrow). × 42,000.

Fig. 16. Cross section of the apical region of the ductule (d) showing the usual location of the microvilliform canal cell plasmalemma (*) within the concavitiy formed by the furrowed ductule. Several maculae adherents (ma) and septate desmosomes (sd) bind the opposing plasmic surfaces of the canal cell. × 20,000.

Fig. 17. During intermolt the ductule (d) of the type A gland appears to be completely collapsed, indicating a temporary cessation of secretory activity. The cross-sectional profiles of the numerous microtubules (mt) that characterize the canal cell cytoplasm are easily recognized in this section. × 25,000.

Fig. 18. Cross section of the newly formed ductule (d) and the preexisting old ductule (od) of a type A rosette gland during late premolt. The enveloping, moderately lucent cytoplasm of the canal cell (CC) appears nearly circular in profile. Notice the presence of a moderately dense secretory material (*) between the old and new ductules; this suggests that the secretions are destined to pass into the ecdysial space. × 20,000.

Fig. 19. Cross section of both the newly formed ductule (d) and the old ductule (od) of a type A rosette gland during late premolt. This demonstrates that in some instances the moderately dense secretory material (*) is contained within the old ductule and is perhaps destined for the branchial surface but not the ecdysial space. × 20,000.



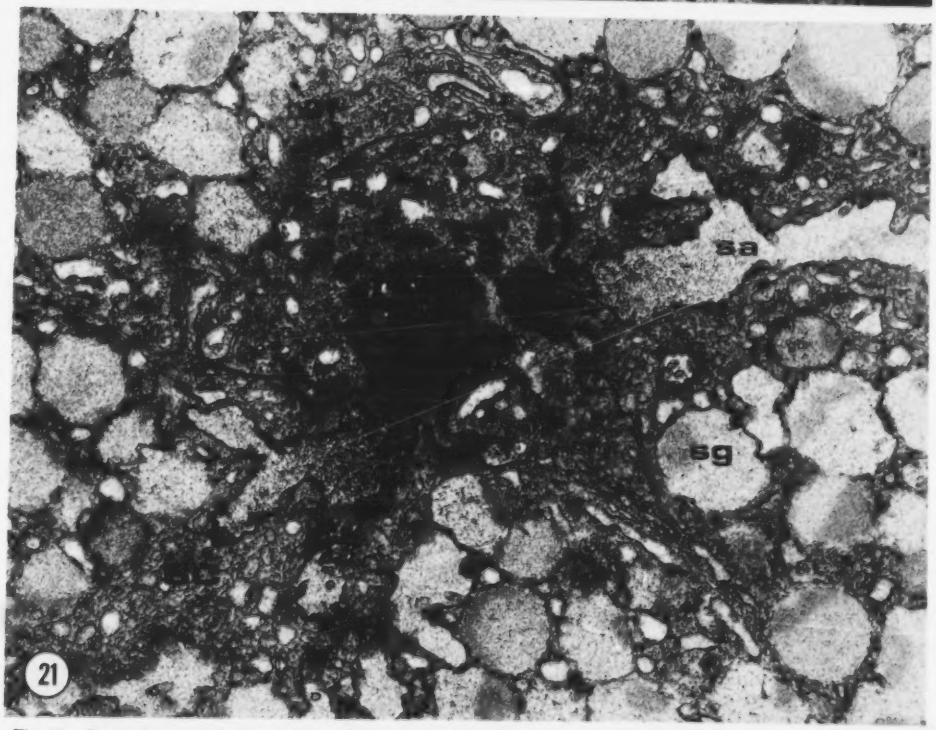
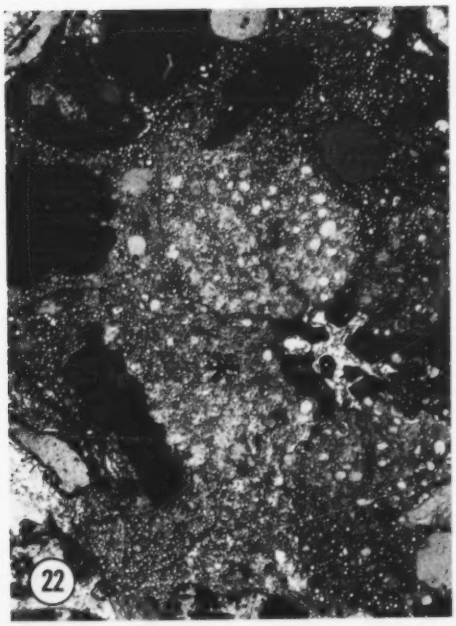
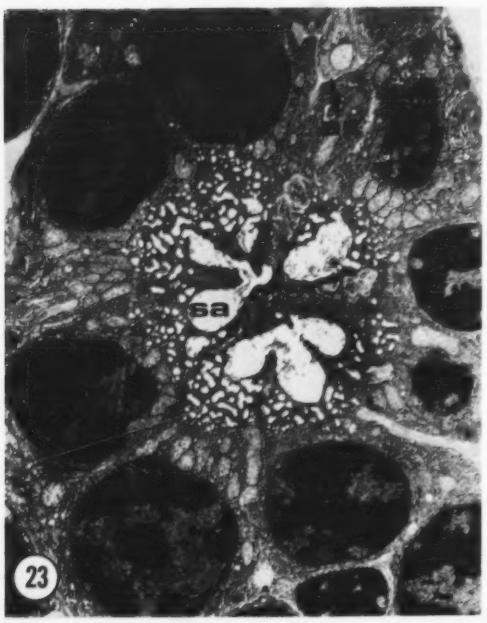
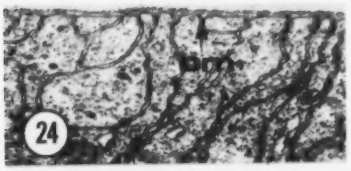


Fig. 20. Sagittal section through the ductule terminus of a type A rosette gland during late premolt. The old ductule (od) formed during the previous premolt period terminates at the surface of the old branchial exoskeleton (obe). The newly formed ductule (nd) terminates at the surface of the newly forming branchial exoskeleton (be). A moderately dense, granular secretion (*) is passing between the old and the new ductules into the ecdysial space (es). × 20,000.

Fig. 21. Section through the central core of a type A rosette gland within 2 hours after ecdysis. The contents of numerous secretory granules are passing through the secretory apparatuses (sa) into the extracellular channels of the central cell (C). An exaggerated morphological transformation in the secretory material density has occurred within the latter. Notice that the secretory material does not leak beyond the septate junctions (arrow) that bind the secretory cells to the central cell. Patches of a dense filamentous and granular material (gf) populate the cytoplasma adjacent to the central cell extracellular channels. × 16,500.







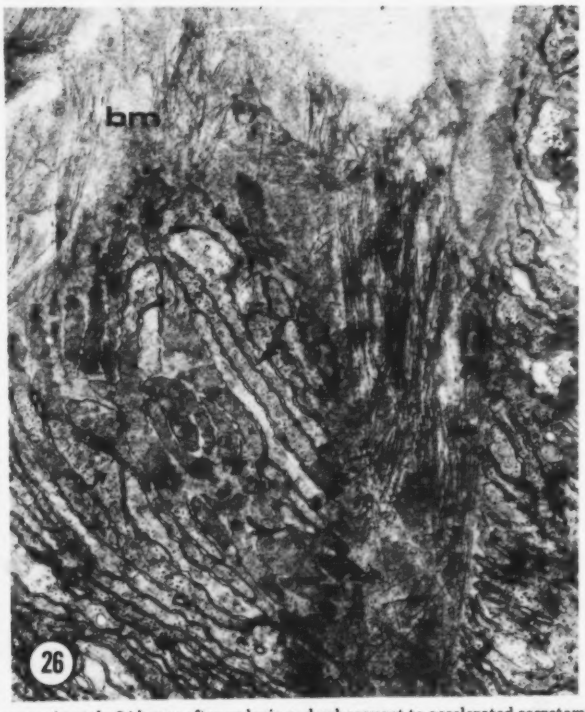


Fig. 22. Overview of type A rosette gland approximately 24 hours after ecdysis and subsequent to accelerated secretory activity. The cytoplasm of the component secretory cells appears relatively disorganized and very few secretory granules are present (*). × 2,000.

Fig. 23. Overview of type B rosette gland during late premolt. The radially arranged secretory cells are characterized by a basal cytoplasm with numerous mitochondria and few cisternae of endoplasmic reticulum. A well-developed secretory apparatus (sa) occupies the apical cytoplasm. Note the nerve axon (ax) which is penetrating the gland. × 2,000.

Fig. 24. Low magnification cross section of the typically thin basement membrane (bm) of the secretory cells of the type B rosette gland. × 15,000.

Fig. 25. High magnification frontal section of the type B rosette gland basement membrane. Notice the generally granular nature (*) of this layer. The surface of a type B gland secretory cell (SC) is also visible. × 45,000.

Fig. 26. Frontal section of a type B rosette gland showing the numerous plasmalemmal grooves (arrows) formed from the superficial invaginations and interdigitations of the secretory cell basal plasmalemmas. Numerous fibrillar elements (f) are embedded within the granular matrix of the basement membrane (bm). × 14,000.

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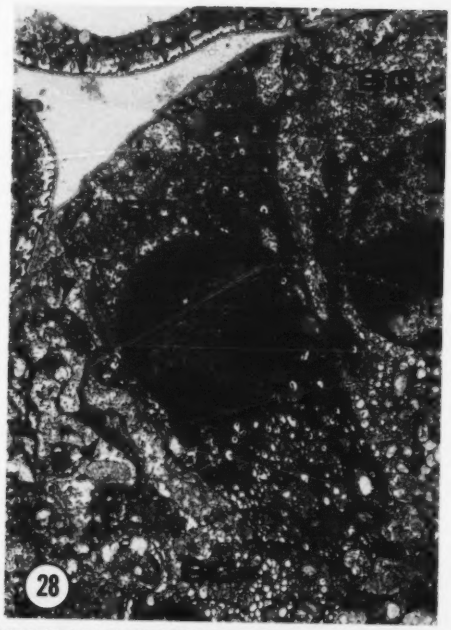
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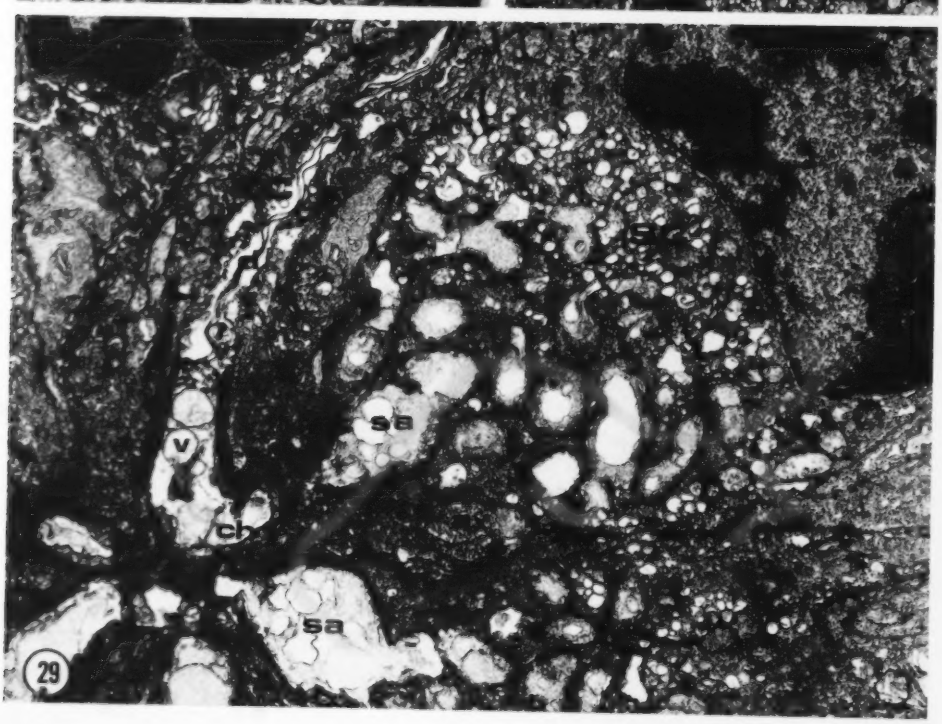
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a usually conspicuous secretory apparatus (Fig. 29), which in serial sections appears to be a highly branched invagination of the cell apex. The system of extracellular channels and pools so formed radiates throughout the apical cytoplasm. Furthermore, as the channels penetrate deeper into the cytoplasm, their luminal dimensions decrease. For the most part the network of TER resides within the cytoplasmic interstices of the extracellular secretory apparatus (Figs. 30, 31). Continuity between the membranes of the TER and the invaginated plasmalemma, which delimits the secretory apparatus, has not been observed. In some instances, the plasmalemma that borders the channels of the secretory apparatus is absent, and the channels are filled with relatively lucent cytoplasmic substances presumed to be secretory products destined to move out of the gland. In these instances the tubular endoplasmic reticulum is in direct contact with the secretory products (Figs. 30, 33). In some cases the elements of TER are ruptured (Fig. 33); whether this is a fixation artifact or a stage in the secretory process remains to be ascertained.

Central cell. The cytoplasm of this cell is bilobed, consisting of a central and peripheral lobe (Fig. 1). The central lobe occupies the core of the gland and envelops a system of extracellular channels (Figs. 1, 29), which are confluent with the secretory apparatuses of the secretory cell. These channels merge at a common cylindrical vent (Figs. 1, 29). The vent is lined with an electron-dense granular intima that is formed by the central cell and originates near the vent base (Fig. 1). The intima gradually increases in thickness until it merges with the epicuticular ductule (Fig. 30), formed and enveloped by the adjacent canal cell. The cytoplasm surrounding the extracellular channels is characterized by occasional Golgi elements, frequent cisternae of RER, and numerous small vesicles with dense matrices. Also, adjacent to the extracellular channels are found sinuous electron-dense bundles composed of both granular and filamentous material (Fig. 30). The peripheral lobe of the central cell extends to the gland periphery and contains a usually oblong nucleus, RER, Golgi, and mitochondria (Fig. 1).

Canal cell. This cell (Fig. 1) wraps around the ductule so as to form an extracellular canal; the opposing plasmic surfaces are joined by a macula adherens and septate desmosomes. The ductule is composed of trilaminated epicuticle and in cross section usually has an expanded U-shaped profile. A cylindrical extension of the canal cell envelops the ductule as it leaves the gland and ascends to the branchial surface. The terminal orifice is usually located at points midway between adjacent gill lamellae and in close proximity to the ductule orifice of the type A rosette gland (Fig. 2). The cytoplasm is relatively lucent and, as in the type rosette gland canal cell, contains abundant microtubules and small (0.1–0.25 μm) vesicles.

Innervation. A large nerve fascicle (ca. 7.5 × 5.0 μm) passes from the cephalothorax into each of the gill axes via the short connecting peduncles (Fig. 2). Cross sections of the nerve fascicle reveal that it is composed of a number of distinct axonal processes which vary in diameter (ca. 0.2–3.0 μm) (Fig. 34). All the processes are initially confined within an outer sheath, composed of interwoven collagen-like fibrils (ca. 200 Å). Inward extensions of this sheath further segregate smaller groups of axonal processes. In addition to small mitochondria and vesicular elements, the axonal processes contain numerous microtubules. The nerve fascicle divaricates at the site where it enters the gill axis, and each branch becomes further ramified as it travels along the longitudinal axis of the gill. At least some ax-

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Fig. 27. Basal cytoplasm of type B rosette gland secretory cell demonstrating the extensively infolded basal plasmalemma adjacent to the efferent hemolymph channel (Ef). Notice the close association between the infoldings and the numerous spherical mitochondria (m). × 8,400.

Fig. 28. Rare instance where adjacent secretory cells of the type B rosette gland are of different cytoplasmic density. This micrograph clearly demonstrates that there is considerable interdigitation (arrows) between adjacent secretory cells. × 3,600.

Fig. 29. Overview of central core of type B rosette gland demonstrating the envelopment of the secretory cell (SC) apices by the central cell (C). The secretory apparatuses (SA) are confluent with the central cell extracellular channels (ch). Secretory materials pass through the central cell vent (v) to the cuticular ductule (d) of the canal cell (CC). Note that the canal cell plasmalemma, associated with the ductule, is microvilliform (arrow). Several axonal processes (ax) occupy the extracellular spaces between the secretory cells and the central cell. The gland is from an intermolt shrimp. × 7,500.

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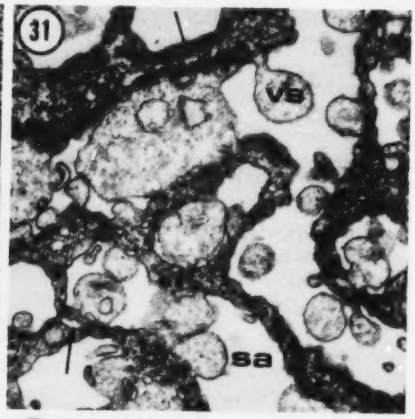
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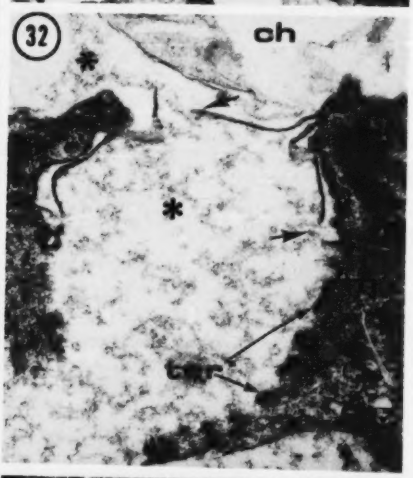
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onal processes terminate within the type B rosette glands. Specifically, a nerve process penetrates the gland via the extracellular spaces between adjacent secretory cells (Figs. 23, 35), toward the gland core (Fig. 36). Usually the penetrating nerve element consists of a single axonal process. Occasionally, however, the penetrating element consists of a bundle of axons (Fig. 37). As the axons approach the tricellular junction between adjacent secretory cells and the central cell, bulb-like extensions of the axoplasm become evident (Figs. 38, 39). These extensions lack a collagen-like sheath and are densely populated with at least two distinct types of vesicles. The larger vesicles (900–1,300 Å) usually possess an electron-dense core surrounded by a lucent halo (Fig. 38). The smaller vesicles (450–640 Å) possess lucent matrices and may be either spherical or flattened (Fig. 40). Synaptic junctions occur between the axoplasmic extensions and the secretory cells of the type B gland. The junctions (1,600–1,700 Å diameter) are characterized by presysnaptic and postsynaptic desmosomid densities (Figs. 40, 41). Typically, the small clear vesicles are congregated around the presynaptic desmosomid density. Synapses between the axonal extensions and the central cell were not observed.

Secretory activity. Secretion in the type B rosette gland is generally affected by exocytosis. However, the secretory activity does not involve the exocytosis of previously formed secretory granules, as in the type A glands. Instead, the plasma membranes delimiting the secretory apparatuses of the type B glands develop a number of polymorphic evaginations; these evaginations frequently appear vesicular, and contain a precipitated material in a lucent matrix (Fig. 31). These vesicular structures, which may have a variable size and shape (Figs. 30, 31), appear to be pinched off into the secretory apparatus (Fig. 31) and then passed into the extracellular channels and the vent of the central cell (Fig. 30). During this passage, the integrity of the vesicular membrane is usually lost or diminished. The forceful movement of secretory substances from the apical cytoplasm of the secretory cell appears to push the limiting plasmalemma of the secretory apparatus into the extracellular channels of the central cell (Fig. 30). In rare instances, the outwardly expanded secretory apparatus plasmalemma showed ruptures (Fig. 32). As mentioned previously, elements of TER are seen around the accumulating secretory materials in the apical cytoplasm of the secretory cell (Fig. 30).

The morphological status of all type B glands in a given animal is nearly identical at any one time, indicating that these glands function in unison. In general, the type B glands appear to be active during intermolt, at the time of ecdysis, and shortly after ecdysis; they seem to display maximal secretory activity during early postecdysial period. The gland cells display molt-related alterations in the morphology of mitochondria, the degree of

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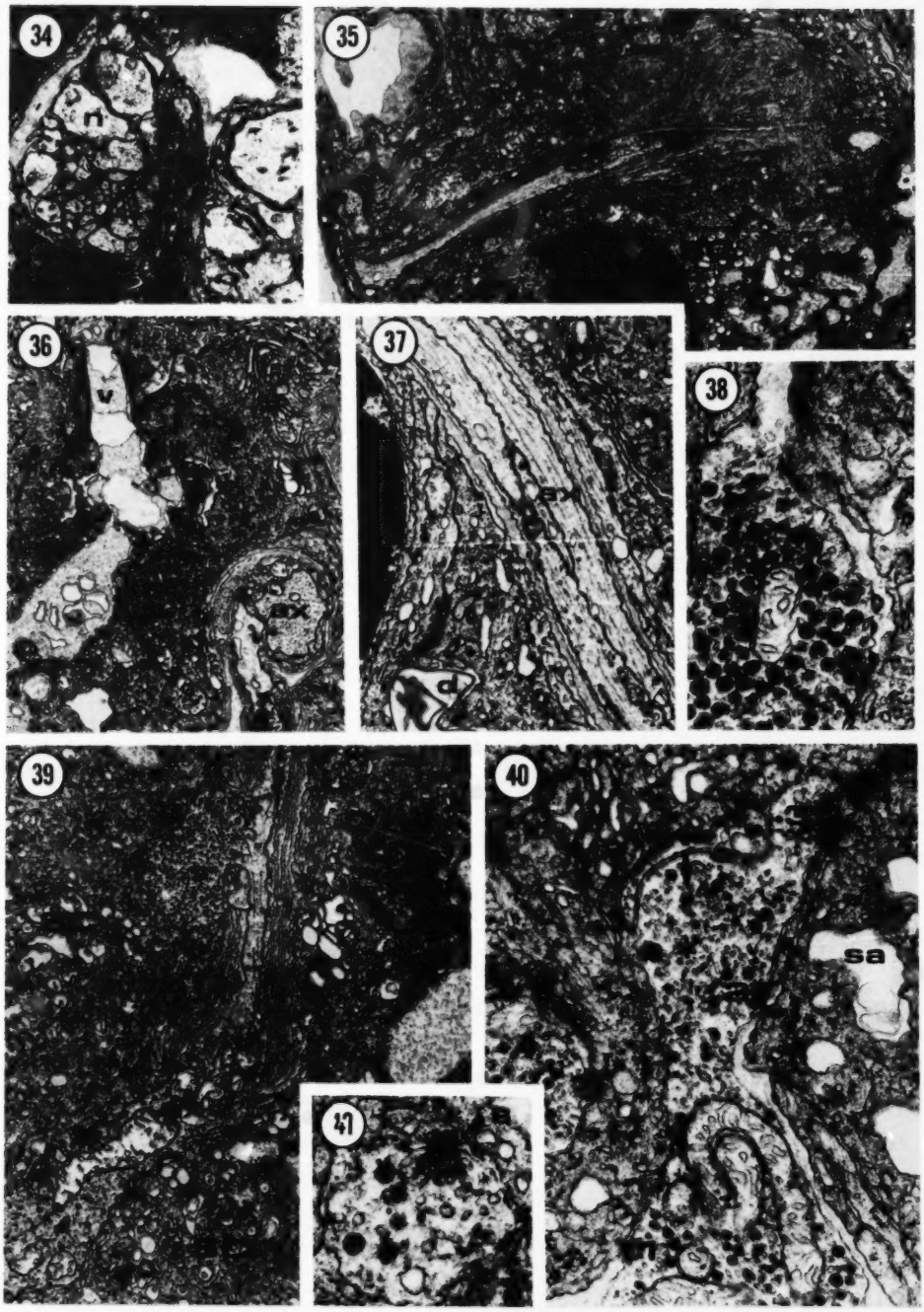
Fig. 30. Section through the core of a type B rosette gland of an intermolt shrimp in which a presumptive, forming secretory vesicle (vs_1) is protruding from the apex of the secretory cell (SC) into an extracellular channel (ch) of the central cell (C). Elements of the network of tubular endoplasmic reticulum (ter), characteristic of the apical cytoplasm of the secretory cell, are seen in direct contact with the secretory products accumulating in the forming vesicle (vs_1). A secreted vesicle (vs_2) is present in the central cell vent (v), which leads to the ductule (d). The intima (i) lining the vent increases in thickness as it approaches the ductule. Note the electron-dense patches of granular and fibrillar material (gf) that characterize the central cell cytoplasm adjacent to the extracellular channels (ch). × 16,000.

Fig. 31. Section through the peripheral region of the secretory apparatus (sa) of a type B rosette gland secretory cell of a late premolt shrimp in which secretion appears to involve the evagination of the delimiting plasmalemma to form fluid-filled vesicles (vs). Note the elements of tubular endoplasmic reticulum (arrows). × 15,000.

Fig. 32. Section through a type B gland of an intermolt shrimp in which the delimiting plasmalemma of the secretory apparatus is ruptured at several sites (arrows). The secretory products (*) of the secretory cell (SC) are passing into the extracellular channel (ch) of the central cell (C). Note the elements of tubular endoplasmic reticulum (ter) and the macula adherens (ma) that adjoin the secretory cell and central cell. × 30,000.

Fig. 33. Cross section through an intermolt shrimp type B rosette gland secretory cell (SC) several microns subjacent to the apex, in the area normally occupied by the tubular, major extracellular channel of the secretory apparatus. The area shown in this section is presumed to be similar to that of the secretory cell apex shown in Figure 30. Circularly arranged elements of tubular endoplasmic reticulum (ter) are found in association with accumulating secretory substances. Notice that some elements of the tubular endoplasmic reticulum are ruptured (arrows). × 25,000.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ramification of the secretory apparatuses, and the types and quantities of secretory materials visible within the channels of the secretory apparatuses.

During intermolt (stage C), the secretory cells are characterized by a usually ovoid or spherical basal mitochondria with moderately lucent matrices and closely spaced cristae (Fig. 42). In addition, the secretory apparatus is moderately extensive and contains a moderately dense flocculent secretion (Fig. 42). During early premolt (stages D_0–D_1) the secretory apparatus is absent. Temporary cytoplasmic processes emerge from the secretory cell apex and occlude the extracellular channels of the central cell (see Doughtie and Rao, 81b); this appears to block effectively any glandular secretion during the formation of epicuticle. The temporary, occluding cytoplasmic processes disappear prior to late premolt (stage D_4) at which time an extensive secretory apparatus with markedly expanded channels is reformed (Fig. 43). During this period the channels of the secretory apparatuses contain an extremely electron-lucent material, and there is no evidence that the plasmalemma of the secretory apparatus channels is engaged in the formation of tethered vesicles. Also during late premolt, the basal mitochondria are generally elongated with the long axes directed toward the center of the gland. The mitochondrial matrices continue to appear moderately lucent, but the cristae are more distantly spaced than during intermolt (Fig. 43). Within 5–10 minutes after ecdysis, the peripheral, narrow channels of the secretory apparatuses are obscure, but the wide apical channels are filled with vesicular elements together with a flocculent-appearing material (Fig. 44). The basal mitochondria are more elongated and contracted than during late premolt, and the matrices appear more electron-dense (Fig. 44, inset). Twenty-four to thirty-six hours after ecdysis, the secretory apparatuses appear relatively reduced in size and contain primarily a moderately dense, precipitated material (Fig. 45) that passes into the ductule. The appearance of the secretory apparatuses and of their contents is similar to that in Type B glands during intermolt (Fig. 30). In contrast to the elongated, condensed appearance of the basal mitochondria soon after ecdysis, the mitochondria of secretory cells 24–36 hours after ecdysis possess dilated, often spherical profiles with sparse cristae and an electron lucent matrix (Fig. 45, inset). Finally, the average size of the type B glands is less (ca. 50 μm) at this time than during all other stages (ca. 70 μm).

An interesting phenomenon associated with most type B glands during the 24–36-hour postecdysial interval is the occurrence of a large, ovoid, membrane-delimited reservoir within the central cell. A very fine filamentous material appears to revolve around a point near the center of the reservoir (Fig. 45).

Fig. 34. Cross section of the nerve fascicle (n) passing through the peduncle from the cephalothorax into the gil axis. × 4,500.

Fig. 35. A nerve axon (ax) penetrating a type B rosette gland through the extracellular space between adjacent secretory cells (SC). × 4,500.

Fig. 36. Section through the core of a type B gland showing the close proximity between a cross-sectioned nerve axon (ax) and the central cell vent (v). × 7,000.

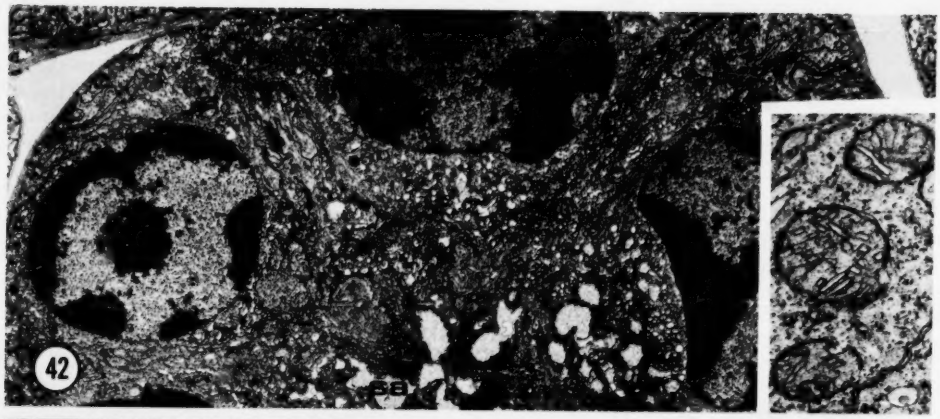
Fig. 37. Sagittal section through a group of nerve axons (ax) passing into a type B gland, near the ductule (d). Notice the typical microtubular and vesicular axonal inclusions. × 10,000.

Fig. 38. High magnification of a bulbous axonal terminal between adjacent secretory cells. The predominant vesicle type in this section is large and possesses a dense core with a lucent halo. × 30,000.

Fig. 39. Axonal process passing between the apices of two type B gland secretory cells (SC) and the central cell (C). An evagination of the axon results in the formation of a terminal (t) filled with two vesicle types. × 30,000.

Fig. 40. High magnification of a nerve terminal near a secretory cell secretory apparatus (sa) in the type B gland. Large, dense-cored vesicles, resembling neurosecretory veiscles (nv), and smaller lucent vesicles, of the synaptic type (*), populate the axon terminal. The smaller synaptic vesicles have congregated near the limiting membrane to form three presynaptic zones (1–3). Zone 3 appears to exhibit a postsynaptic desmosomid density as well. × 20,000.

Fig. 41. High magnification of axon synapse with the secretory cell of a type B rosette gland. Both pre- and postsynaptic desmosomid densities are evident. × 34,000.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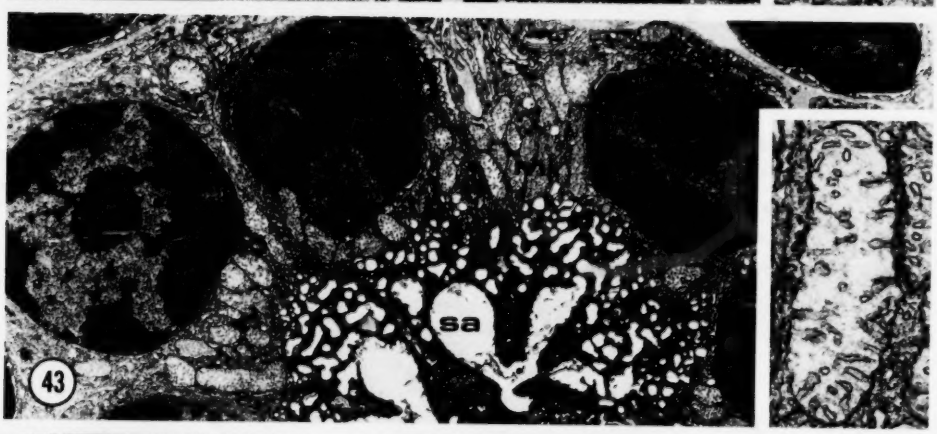
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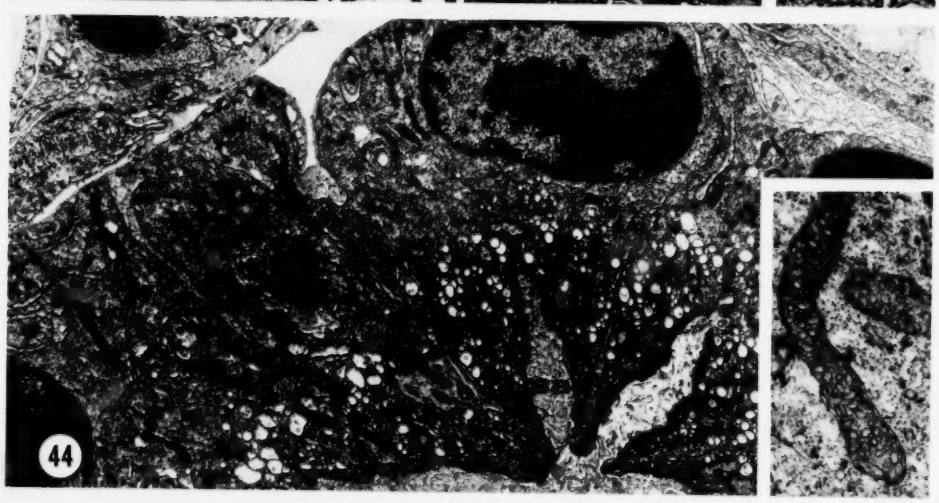
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Whether the contents of this reservoir are derived from the central cell, or from the secretory cells is not known. No definitive connection between the extracellular channels of the central cell and the reservoir was observed.

General morphology of immature, type A and type B rosette glands

Immature type A and type B branchial rosette glands are relatively uncommon in the gills of larger (24–28 mm, total length) grass shrimp. On the other hand, the majority of the rosette glands occurring in the gills of smaller (14 –18 mm, total length) grass shrimp appear underdeveloped. Specifically, the immature rosette glands typically possess fewer secretory cells (< 11 in type A gland; < 8 in type B gland). Furthermore, the immature glands are rarely spherical or ovoid, but usually conform to the irregular profiles of the surrounding tissues. The rarely conical secretory cells in immature type A rosette glands are characterized by a relative paucity of both secretory granules and elements of basal rough endoplasmic reticulum (Fig. 46), even at stages in the molt cycle when mature glands are characterized by numerous secretory granules (see Fig. 3). Similarly, the secretory cells of immature type B glands lack an extensively infolded basal plasmalemma, and the basal cytoplasm contains relatively few mitochondria. In both the immature type A and Type B glands, the ductule passing through the canal cell usually possesses a cylindrical lumen (Fig. 47) in contrast to the expanded U-shaped lumen that characterizes the ductule of mature glands (see Fig. 16). Furthermore, the luminal dimensions of the vent and the epicuticular ductule (0.3–0.6 μm diameter) are less than in mature glands, and increase in size during each succeeding molt (Fig. 48).

DISCUSSION

Since the main focus of this study is on the fine structural features of the well-developed (mature) rosette glands of *Palaemonetes*, all but the last section of the discussion pertains to mature glands.

Comparative morphology of rosette glands

The general organization of the type A and type B rosette glands of *Palaemonetes pugio* closely parallels the histological descriptions of rosette glands in other crustaceans (e.g., Farkas, '27; Yonge, '32; Gorvett, '46; Stevenson, '61, '64). The unifying characteristics include 1) an ovoid or spherical profile, 2) the presence of numerous radially arranged secretory cells, and 3) the presence of several accessory cells that ensheath various regions of an exocrine ductule, leading to the cuticular surface. Additionally, it is probable that the type A and type B glands are homologous to the "reticulate gland" and "clear gland," respectively, described for the gills of *Palaemonetes varians* (Allen, 1893). Since the rosette glands (types A and B) of *Palaemonetes pugio* possess both secretory cells and accessory cells, one of which envelops a cuticular ductule, they may be equated with the class III-type exocrine glands of insects, as defined by Noirot and Quennedy ('74).

The ultrastructural features of the rosette glands of *Homarus* (Arsenault et al., '79) are strikingly similar to those of the type A gland described here. Excepting that the former gland may possess as many as fifty secretory cells, the similarities are best reflected in the nearly identical cell-to-cell interrelationships. Furthermore, both glands possess patches of a dense granular and filamentous material in association with the extracellular channels of the central cell. This material, also present

Fig. 42. Section through a type B rosette gland, representative of the component secretory cells during intermolt (stage C) and premolt (stages D_1–D_2). The basal mitochondria (m) are spherical, the secretory apparatus (sa) is moderately defined, and a moderately dense precipitate-like material is present within the secretory apparatus. × 3,200. Inset: High magnification of the basal mitochondria. × 20,000.

Fig. 43. Section that is representative of the morphology of type B rosette gland secretory cells during late premolt (stages D_3–D_4). The secretory aparatus (sa) is extensively developed and contains only an electron-lucent material. The basal mitochondria (m) are elongated with moderately lucent intercristal matrices. × 3,000. Inset: High magnification of basal mitochondria. × 20,000.

Fig. 44. Section through a type B rosette gland within 5–10 minutes after ecdysis, showing the characteristics of the component secretory cells. The peripheral channels of the secretory apparatuses are obscure but the apical channels (sa) are generally occluded by vesicular elements and a precipitated substance. The mitochondria (m) are elongated and appear electron-dense, owing to their condensed state. × 3,000. Inset: High magnification of representative mitochondria. × 20,000.

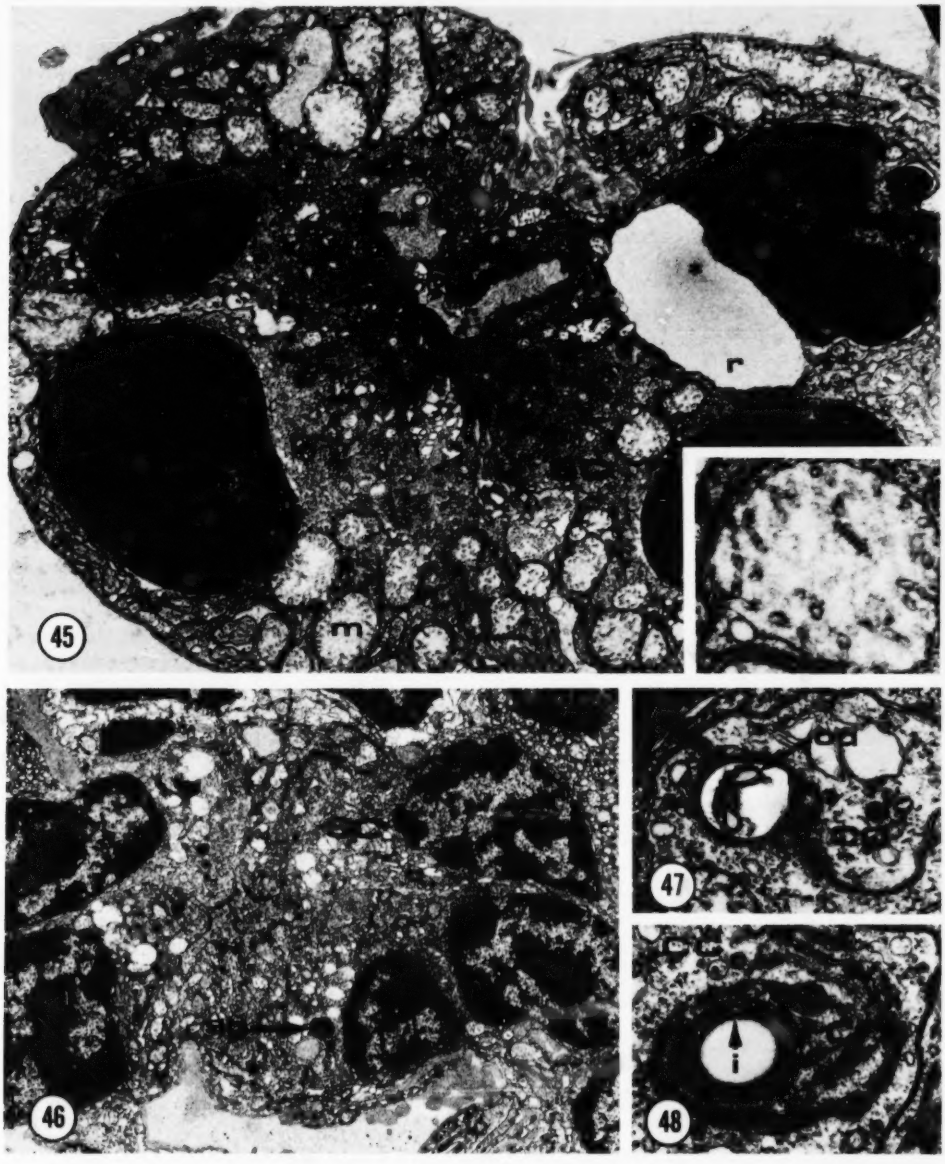

Fig. 45. Section through a type B rosette gland 24–36 hours after ecdysis. The gland is smaller than during other stages. The secretory apparatuses (sa) of the component secretory cells (SC) are poorly defined and contain a moderately electron-dense, precipitate-like material. The mitochondria (m) are distended and spherical with sparse cristae and lucent matrices. A large fluid-filled reservoir (r) occupies the central cell (C) cytoplasm. Close observation shows that a precipitated material appears to rotate around a common center (*). × 4,500. Inset: High magnification of representative mitochondria. × 20,000.

Fig. 46. Overview of immature type A rosette gland. Notice the relatively small number of component secretory cells (SC), and the general absence of secretory granules (sg). There is also a poorly developed network of rough endoplasmic reticulum in the basal cytoplasm. × 3,000.

around the locular complex of the tricellular dermal gland (Doughtie and Rao, '79), presumably functions in the maintenance of channel integrity (Arsenault et al., '79; Doughtie and Rao, '79). Whereas the secretory cells of the rosette glands in *Homarus* and those of the type A gland in *Palaemonetes* possess cytoplasmic elements indicative of a high degree of synthetic capability (i.e., extensive RER, abundant Golgi, and numerous secretory granules), the secretory cells of the type B glands do not; instead, they show characteristics of transport epithelia. Furthermore, unlike the type A glands and all other previously described crustacean exocrine glands, the type B glands are innervated and have neuronal synapses. These special features are discussed below.

Type B gland secretory cells: Features of transport epithelia

Among the noteworthy features of the type B gland are 1) the occurrence of an extensive system of extracellular channels formed by deep infoldings and interdigitations of the basal plasmalemma of the secretory cell surfaces facing the hemolymph, 2) the association of numerous mitochondria with these plasmalemmal infoldings and interdigitations, and 3) the presence of a network of tubular smooth endoplasmic reticulum in the apical cytoplasm of the secretory cells. Since these features are known to be characteristic of epithelia involved in salt-ion transport (see Diamond and Tormey, '66; Diamond and Bossert, '67, '68; Philpott and Copeland, '63; Conte, '69), including the salt glands of birds (Peaker and Linzell, '75), it is tempting to speculate that the type B rosette glands in grass shrimp gills may have an osmoregulatory function.

Interdigitating subcuticular epithelial cells, which, like the secretory cells of the type B gland, are characterized by a deeply infolded plasmalemma and numerous associated mitochondria, have been described in the gills of the crabs *Gecarcinus* (Copeland, '68) and *Callinectes* (Copeland and Fitzjarrel, '68), of the crayfish *Astacus* (Bielawski, '71) and of the shrimp *Penaeus* (Foster and Howse, '78). These cells are generally believed to participate in osmoregulation. However, exocrine glands that are composed of secretory cells with similar characteristics have not been described previously in either the gills or other subcuticular tissues of a crustacean.

Innervation of type B glands

Another characteristic feature of these glands is the occurrence of innervation. Except for a recent finding of a nerve fascicle in the gill axis of *Penaeus* (Foster and Howse, '78), nothing is known of the sites of innervation in crustacean gills. Furthermore, except for a report on the occurrence of nerve fibers adjacent to a unicellular tegumental gland in the mystacocaridean crustacean, *Derocheilocaris* (Pochon-Masson et al., '75), there have been no previous reports on the innervation of crustacean tegumental glands.

Actually, even in the other arthropods (insects, arachnids, etc.), the innervation of exocrine glands is considered to be a rare phenomenon (see review by Noirot and Quennedy, '74). In the few reported cases (Gupta and Smith, '69; Quennedy, '69; Whitehead, '71; Plattner et al., '72; Kovoor and Zylberberg, '79), the most frequently seen vesicular inclusions in the nerve terminations are the larger (900–2,000 Å) granular vesicles; less frequently seen are the smaller (400–650 Å) agranular vesicles (Quennedy, '69; Whitehead, '71). The larger granular vesicles are thought to have a neurosecretory function (e.g., Plattner et al., '72); the smaller vesicles are considered to be of the synaptoid type, although postsynaptic differentiation is not demonstrable in most cases (see Noirot and Quennedy, '74). Both the granular and agranular vesicles occur in the nerve terminations associated with the secretory cells of the type B gland in *Palaemonetes*. Furthermore, postsynaptic differentiation appears to exist at the plasma membrane of the associated type B gland secretory cells (see Figs. 40, 41). The full significance of the innervation of these glands can be appreciated only when the function of the glands can be ascertained by further studies.

Fig. 47. Cross section of more distal region of immature rosette gland ductule within the canal cell (CC) during late premolt. The ductule diameter is considerably less than that of mature glands. Notice the old ductule (od) within the lumen of the reformed ductule, and the greater size of the latter. × 20,000.

Fig. 48. Cross section of fibrous intima (i), which lines the vent of the rosette gland central cell (C). The diameter of the vent in this immature rosette gland is considerably less than in mature glands. Canal cell (CC). × 18,000.

Secretory activity and functional correlations

Type A rosette glands. The type A glands function in unison and, as in the rosette glands of *Homarus* (Arsenault et al., '79), exhibit cyclical activity in accordance with stages in the molt cycle. During intermolt, secretory activity is variable and sporadic; both the rosette glands of *Homarus* and the type A gland exhibit a virtual cessation of secretory activity during early premolt. In *Palaemonetes,* we propose that this is due to the occlusion of the ductule by numerous cytoplasmic processes that are associated with ductule reformation (Doughtie and Rao, '82). The type A gland is active during late premolt (secretion externalized to ecdysial space) and displays maximal activity shortly after ecdysis (secretion externalized to the branchial surface). The force that perpetrates secretion in this gland is not entirely clear. It is likely, however, that increased hydrostatic pressures of the hemolymph, resulting in gland compression and secretion, occur at ecdysis as the shrimp's body contracts repetitively in an attempt to slough off the old exoskeleton. Similarly, the postecdysial secretions may also result from positive hemolymph pressures caused from the rapid uptake of fluids as the shrimp attempts to expand the newly formed exoskeleton (see Lockwood, '77).

The rosette type glands of Crustacea have been implicated in the secretion of phenol oxidase, an enzyme responsible for cuticular tanning (e.g., Krishnan, '51; Stevenson, '61). This enzyme appears to be released maximally during the period shortly after ecdysis (Krishnan, '51). Since the type A glands of *Palaemonetes,* like the rosette glands of *Homarus* (Arsenault et al., '79), contain secretory granules and exhibit maximum secretory activity during the early postmolt period, it would be worthwhile to determine whether they are engaged in the synthesis and secretion of phenol oxidase. The application of histochemical tests would be useful in this respect.

The present study and other recent reports (Arsenault et al., '79; Elofsson et al., '78) indicate that large, membrane-bound vesicles or granules characterize the secretory cell cytoplasm in the majority of described rosette glands. In certain crustacean exocrine glands, the secretory granules are shown to originate from the Golgi apparatus (Rieder, '77; Juberthie-Jupeau and Crouau, '79); but in the integumentary glands of a copepod, the secretory "progranules" appeared to be formed by the rough endoplasmic reticulum (Gharagozlu-van Ginneken, '79). In the type A gland of *Palaemonetes,* whereas some of the secretory granules appear to arise from the Golgi bodies (Figs. 3, 4), many of the secretory granules seem to originate (partly or solely) from both the smooth and rough endoplasmic reticulum (Figs. 6–8). Although more critical studies are needed to elucidate the relative contribution of the different organelles to granule formation, the available evidence suggests that, whereas the Golgi bodies help elaborate secretory granules throughout most stages of the molt cycle, the endoplasmic reticulum participates in granule formation during premolt. The granules from both sources appear to be externalized during late premolt and shortly after ecdysis.

The contribution of the endoplasmic reticulum to granule formation merits additional discussion. Until recently, it was generally believed that the Golgi bodies were a mandatory link in the synthesis and exportation of substances out of eucaryotic exocrine cells (see Palade, '75; Jamieson and Palade, '77). However, it has been shown that glycosylation, a process normally associated with the Golgi apparatus, can occur within the rough endoplasmic reticulum (Pelletier and Puviani, '73). Furthermore, recent studies of a wide range of animals have suggested that substances destined for extracellular export may be formed partly or solely by the endoplasmic reticulum (e.g., Ross and Benditt, '65; Ashhurst and Costin, '76; Busson-Mabilliot, '77).

Type B rosette glands. On the basis of evidence presented here (Figs. 29–33; 42–45), the type B glands appear to be active during intermolt, molt (ecdysis), and early postmolt stages. Secretory activity may also occur during late premolt, but a visible secretory product was not apparent in the secretory apparatuses at that time. There is a virtual cessation of secretory activity during early premolt, due to ductule blockage by transitory cytoplasmic processes (see Doughtie and Rao, '82).

Despite these general indications of secretory activity, the implications of the more obvious changes (i.e., mitochondrial profiles, degree of secretory apparatus elaboration, and the type of material present within the secretory apparatuses) are not well understood. The morphological transformations that occur within the secretory cell mitochondria over the course of the molt cycle probably reflect changes in their respiratory state or metabolic

activity. For example, in a number of previous histophysiological studies, the morphologies of isolated rat mitochondria have been linked to their respiratory state (e.g., Muscatello et al. '72, '75; Hackenbrock, '72). Furthermore, in the glands of spiders, visible changes in mitochondria have been used as morphological indices of assessing changes in gland activity (Komnick, '63; Kovoor and Zylberberg, '79). It is generally believed that mitochondria in the contracted state reflect periods when ATP utilization has increased. This being the case, it is possible that the type B rosette glands are engaged in increased active transport of hemolymph substances soon after ecdysis (see Fig. 44). This presumption may be reinforced by the apparent relative increase in the amount of visible secretions within the secretory apparatuses at this time. On the other hand, the absence of a visible (i.e., electron-dense) material in the secretory apparatuses of the type B gland prior to ecdysis (see Fig. 43) may be due simply to the fact that the secretions, if present, are electron-lucent. Additonal studies are required in order to assess the amplitude of the different molt-related secretory activities.

As discussed earlier, the type B glands show characteristics of transport epithelia and presumably participate in ion and water translocation. Perhaps relevant to this point is the fact that other crustacean ion-transporting tissues, particularly the midgut and hindgut, have been shown to be innervated and are presumed to be under neurosecretory control (e.g., Mykles, '79; Holliday et al., '80). Since all of the animals utilized in the present investigation were maintained at a constant, nearly isosmotic salinity, it would be of interest to study these glands in shrimp maintained in hypo- or hyperosmotic media.

Role of accessory cells in secretion modification

In both the type A and type B rosette glands, the central and canal cells may participate in transforming or storing the secretions of the secretory cells. For example, immediately after ecdysis, the materials released from the secretory cells of the type A gland appear to undergo a modification, as indicated by the pronounced changed in electron density, as they pass through the central cell, extracellular channels and into the cuticular ductule (see Figs. 8, 15). It has been suggested that the intercalary (central) cell of insect class III exocrine glands may be involved in the biochemical modification of glandular secretions (Eisner et al., '64; Happ, '68; Lai-Fook '70, '72; Noirot and Quennedy, '74). However, the conspicuous postecdysial transformation of type A gland secretions, described in this report, is the first purely morphological evidence that the central cell functions in the chemical and/or physical modification of secretions from crustacean exocrine glands.

In the type B gland, a large membrane-delimited reservoir, which contains a moderately lucent and seemingly "rotating" fluid, can be seen within the central cell (see Fig. 45) during the early postecdysial period (24–36 hours).

Comparable reservoirs, presumed to function as temporary storage sites or mixing chambers, have been described for the central cell of some insect class III glands (Lai-Fook, '70, '72, '73). However, similar chambers were not apparent in the type A gland and have not been reported for any other crustacean exocrine gland.

The microvilliform elaboration of the canal cell plasmalemma, which is associated with the cuticular ductule of both the type A and type B rosette glands, may be yet another site where the modification of glandular secretions occurs. It is possible that the elaborated folds or microvilli-like processes may function in the dehydration/concentration of the secretion as it ascends through the ductule to the cuticular surface. Although a comparable region was not described for the rosette gland of *Homarus* (Arsenault et al., '79), it has been demonstrated in the tricellular exocrine glands of *Palaemonetes* (Doughtie and Rao, '79) and Antromysis (Juberthie-Jupeau and Crouau, '79).

Changes associated with gland maturation

Although we have not studied the morphogenesis of rosette glands in the gills of *Palaemonetes*, it is clear that a number of distinct changes are associated with gland maturation. Specifically, there is an increase in 1) the number of component secretory cells, 2) the size (diameter) of the ductule, and 3) the degree of specialization of secretory cells, i.e., in synthetic capability, as seen in type A glands, or in plasmalemmal elaborations, as seen in type B gland cells. These characters seem to indicate that the secretory capabilities of underdeveloped glands are considerably less than in the mature glands. However, the physiological demands on these glands are probably commensurately less in the smaller (14–18 mm, total length) shrimp, in which underdeveloped rosette glands constitute a

relative majority of the total rosette gland population. During the incremental molt-related increases in the size of grass shrimp, there is also an increase not only in the length of the gill axes but also in the size and number of lamellar plates (unpublished data). These overall increases in tissue volume and cuticular surface area are certainly accompanied by equivalent increases in the secretory capabilities of the rosette glands. These increases seem to occur not only through the maturation of preexisting glands but also through the morphogenesis of new glands. The latter would account for the relative scarcity of underdeveloped glands in larger (24–28 mm, total length) shrimp. The rare occurrence of underdeveloped rosette glands in the gills of larger shrimp may also be related to periodical replacement of older rosette glands. This phenomenon has been described previously for the rosette glands of *Homarus* (Arsenault et al., '79). In fact, it has been shown that, subsequent to ecdysis, the branchial rosette glands of *Palaemonetes* are sometimes phagocytosed by wandering podocytes (Doughtie and Rao, '81).

One of the more interesting changes associated with the maturation of rosette glands is related to the different strategies employed for ductule reformation. Transitory ciliary processes are associated with ductule reformation in underdeveloped glands, whereas transitory microvilli-like processes occur in mature rosette glands. These differences are described in the second paper of this series (Doughtie and Rao, '82).

ACKNOWLEDGMENTS

This study was part of a project supported by grant CR-807417 from the U.S. Environmental Protection Agency. We are thankful to Dr. Thomas Duke, Gulf Breeze Environmental Research Laboratory, for his support and encouragement during this investigation.

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